Experimental head injury in the rat

Part 2: Regional brain energy metabolism in concussive trauma

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A standardized model of acceleration concussion in the rat was used for the study of cerebral energy metabolism during the acute concussive reaction. Impact velocities of 7 and 9 m/sec were used, and the cerebral metabolic state was determined 1, 4, and 15 minutes after the impact. A concussive response could be sustained with a normal energy state in the tissue, but with the more intense reaction to a 9 m/sec impact, energy depletion usually occurred. At 1 minute these changes were most pronounced in the brain-stem regions. At 4 minutes the reactions were more varied but a progression usually occurred during this time, while at 15 minutes restitution was indicated. Hypoxia due to neurogenic pulmonary edema aggravated the state. The findings are compatible with a high metabolic rate during concussion, but progressive changes indicate the rapid appearance of complicating factors, including hypoxemia and probably also ischemia.

KEY WORDS • experimental concussion • hypoxia • ischemia • regional cerebral energy metabolism

According to classical definitions, cerebral concussion is a mechanically induced paralytic or excitatory change of neuronal function, leading to a sudden and short-lasting loss of consciousness without detectable brain damage. However, since morphological changes occur in connection with experimental concussion as well as clinical concussion, the concept has been extended to include various types of focal or general tissue damage. In addition, when considering the pathophysiology of concussion, one has to take into account systemic respiratory and circulatory changes and local effects on the cerebrovascular system. Analyzing a model of concussive acceleration trauma in the rat, we concluded that it is justified to consider concussion in terms of functional effects of mechanical stress upon neuronal tissue, whatever the physico-chemical basis of this reaction may be. On the other hand, the immediate posttraumatic course might well be influenced by the effects of hypoxemia, blood pressure change, cerebral vasomotor reactions, and primary tissue damage.

Both the primary concussion response and the various complicating factors involved may affect cerebral energy metabolism. On one hand there may be a primary change in energy consumption due to altered function,
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while on the other hand impairment of energy production could arise due to tissue hypoxia or ischemia. So far, there are but few studies concerned with energy metabolism in concussive head trauma. Gurdjian, et al., found signs of energy depletion and lactic acidosis only in contused brains. Meyer, et al., calculated the lactate production from arteriovenous differences and cerebral blood flow determinations and observed increased lactate production in contusion injuries. Oxygen consumption increased transiently in light concussion. Nelson, et al., using compression trauma in mice, found evidence of increased metabolic rate during the immediate response to trauma. In clinical studies, acidosis due to increased lactate production was found in the cerebrospinal fluid of patients with severe head injury. None of these studies, however, gives information about the immediate metabolic response involved in concussion due to impact acceleration.

We have used the trauma model described earlier to study the energy metabolism of the rat brain in terms of regional metabolic state determined 1, 4, and 15 minutes after concussive impacts of 7 and 9 m/sec. These levels of trauma induce concussive responses of different degrees. Since complicating factors such as arterial hypoxia are involved, especially in the 9 m/sec trauma, an attempt was made to evaluate their influence.

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**Materials and Methods**

**Experimental Technique**

Male Wistar rats weighing 350 to 400 gm were used. The animals had free access to pellet food and water until the start of the experiments. Anesthesia was induced with 2% to 3% halothane in 75% nitrous oxide and 25% oxygen in a jar, followed by 1% halothane inhaled through a mask while the animal was intubated endotracheally. One femoral artery was cannulated for blood pressure recording and anaerobic sampling of arterial blood for determinations of oxygen pressure (pO2), carbon dioxide pressure (pCO2) and pH. After the operation, lasting less than 10 minutes, the animal was paralyzed with suxamethonium chloride (Celocurin), given intrarrectially, and artificially ventilated with 75% nitrous oxide and 25% oxygen. The rat was placed on its back in a plaster bed to receive the concussive impact. A steady state of respiration was maintained for 30 minutes. Arterial carbon dioxide pressure (PaCO2) was kept within a range of 35 to 42 mm Hg, PaO2 at 100 to 130 mm Hg and temperature at 36.6° to 37.5°C. The arterial blood pressure was continuously monitored.* Acceleration concussion was elicited according to the model described earlier. The occipital crest was hit from below by a piston, accelerating the head and neck in a movement of translation-rotation and retroflexion. Impact velocities of 7 and 9 m/sec were used. The skull coverings were intact (preparation for tissue freezing was not done until after the trauma). Artificial ventilation was maintained during the trauma through very light tubing to minimize its influence on the movement of the head. The concussive effect of the trauma was estimated from the effect on blood pressure.

**Brain Sampling**

The brain was frozen in situ after turning the animal from the supine to the prone position. The conventional technique of pouring liquid nitrogen into a plastic funnel attached to the skull was modified as follows to allow more rapid preparation and a minimal freezing distance to the brain-stem region. A longitudinal skin incision was made over the skull and neck, and the neck muscles were divided and folded away from the occipital bone and dorsal parts of the spine. The skin was lifted to form a funnel, into which the liquid nitrogen was poured. In the concussion groups, the freezing was started at 1, 4, or 15 minutes after the trauma. An arterial blood sample was taken 15 seconds after the start of the freezing for measurements of pO2, pCO2 and pH. When the whole head was frozen (after about 3 minutes) the animal was disconnected from the respirator, and the head and upper body were immersed in liquid nitrogen. The frozen brain, including the cerebellum and brain stem, was chiselled out during intermittent irrigation with liquid nitrogen. The samples were stored at −80°C until analyzed.

*Mingograph used for monitoring arterial blood pressure manufactured by AB Elema-Schönander, Röntgenvägen 2, S-171 95 Solna, Sweden.
Physiological posttrauma data of total series*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>Duration of BP Response (sec)</th>
<th>Physiological Parameters at Freezing Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Temp (°C)</td>
</tr>
<tr>
<td>control</td>
<td>10</td>
<td>–</td>
<td>36.7 ± 0.2</td>
</tr>
<tr>
<td>trauma 7 m/sec</td>
<td></td>
<td></td>
<td>36.7 ± 0.1</td>
</tr>
<tr>
<td>normoxia</td>
<td>1 min</td>
<td>6</td>
<td>40 ± 4</td>
</tr>
<tr>
<td></td>
<td>4 min</td>
<td>6</td>
<td>45 ± 7</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>4</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>hypoxia</td>
<td>1 min</td>
<td>2</td>
<td>50 to 60</td>
</tr>
<tr>
<td></td>
<td>4 min</td>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td>trauma 9 m/sec</td>
<td></td>
<td></td>
<td>36.8 ± 0.1</td>
</tr>
<tr>
<td>normoxia</td>
<td>1 min</td>
<td>6</td>
<td>87 ± 8</td>
</tr>
<tr>
<td></td>
<td>4 min</td>
<td>6</td>
<td>82 ± 6</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>6</td>
<td>77 ± 12</td>
</tr>
<tr>
<td>hypoxia</td>
<td>1 min</td>
<td>5</td>
<td>91 ± 9</td>
</tr>
<tr>
<td></td>
<td>4 min</td>
<td>4</td>
<td>93 ± 8</td>
</tr>
</tbody>
</table>

*Data are given as mean values = SEM, except for hypoxic animals injured with 7 m/sec velocity injury.

Analytical Techniques

The brains were dissected in a refrigerated box at −22°C. Three regions were chosen for the analysis: the occipitoparietal cortex, the dorsal part of the pons, and the medulla oblongata. After the tissue samples were weighed, they were extracted by grinding with glass rods in tubes containing HC1-methanol. Perchloric acid was then added (at 0°C), and the samples were centrifuged. The extracts were analyzed for glucose, phosphocreatine (PCr), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), lactate, and pyruvate, by means of fluorometric enzymatic techniques. The extraction procedure, and the fluorometric analyses, were those of Lowry and Passonneau. Details of techniques have been described previously.

Statistical Methods

In summarizing the results of the different groups and regions, mean and standard error of the mean (SEM) values are usually given. However, since pathological changes could not be expected to follow a normal distribution, the non-parametric Mann-Whitney U test was used for comparison between groups. The paired t-test was used for estimating regional differences of the pathological response in the animals within the different groups.

Results

Physiological and Pathophysiological Variables

The material used for analysis of tissue metabolites is summarized in Table 1, which lists the duration of the reaction of blood pressure to the trauma, and posttrauma body temperature, mean arterial blood pressure (MABP), and arterial blood gas tensions and pH at the time of freezing. The control group consisted of 10 animals. The main experimental groups included animals in which arterial blood gases were not influenced by pulmonary edema (normoxic groups). Some of the experimental animals were hypoxic at the time of freezing, due to pulmonary edema. Four animals were excluded from the material: one animal in the 7 m/sec injury group had no response to concussion, and three animals in the 9 m/sec injury group, which had pulmonary edema, did not survive long enough for freezing at 15 minutes.

The reactions to trauma and the frequency of complicating respiratory failure were comparable to the series previously reported. The gross morphological changes noted were subarachnoid hemorrhage (SAH) in about 50% of the 7 m/sec injury groups and in about 90% of the 9 m/sec injury groups. Macroscopic intracerebral lesions were looked for when the brains were cut for the extraction, but were not found. The body temperature,
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**TABLE 2**

Regional metabolic state (µmol/gm) and lactate/pyruvate ratio in control series and after concussive head trauma with 7 m/sec impact*  

<table>
<thead>
<tr>
<th>PCr</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>La</th>
<th>Py</th>
<th>La/Py</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (10 rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>4.76 ± 0.07</td>
<td>2.96 ± 0.03</td>
<td>0.29 ± 0.01</td>
<td>0.03 ± 0.001</td>
<td>1.49 ± 0.05</td>
<td>0.11 ± 0.006</td>
</tr>
<tr>
<td>pons</td>
<td>4.18 ± 0.16</td>
<td>2.62 ± 0.04</td>
<td>0.23 ± 0.01</td>
<td>0.03 ± 0.003</td>
<td>1.33 ± 0.06</td>
<td>0.07 ± 0.003</td>
</tr>
<tr>
<td>medulla</td>
<td>4.32 ± 0.09</td>
<td>2.41 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>0.03 ± 0.003</td>
<td>1.08 ± 0.05</td>
<td>0.08 ± 0.002</td>
</tr>
<tr>
<td>trauma 1 min (6 rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>4.66 ± 0.08</td>
<td>2.97 ± 0.04</td>
<td>0.28 ± 0.02</td>
<td>0.03 ± 0.006</td>
<td>1.50 ± 0.15</td>
<td>0.10 ± 0.015</td>
</tr>
<tr>
<td>pons</td>
<td>3.99 ± 0.12</td>
<td>2.53 ± 0.02</td>
<td>0.25 ± 0.01t</td>
<td>0.04 ± 0.006</td>
<td>1.39 ± 0.06</td>
<td>0.07 ± 0.009</td>
</tr>
<tr>
<td>medulla</td>
<td>3.89t ± 0.19</td>
<td>2.42 ± 0.02</td>
<td>0.24 ± 0.02t</td>
<td>0.02 ± 0.002</td>
<td>1.76t ± 0.14</td>
<td>0.08 ± 0.003</td>
</tr>
<tr>
<td>trauma 4 min (6 rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>4.68 ± 0.06</td>
<td>2.93 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.03 ± 0.002</td>
<td>1.39 ± 0.09</td>
<td>0.10 ± 0.007</td>
</tr>
<tr>
<td>pons</td>
<td>3.90 ± 0.11</td>
<td>2.58 ± 0.02</td>
<td>0.26 ± 0.02t</td>
<td>0.03 ± 0.007</td>
<td>1.33 ± 0.07</td>
<td>0.06 ± 0.004</td>
</tr>
<tr>
<td>medulla</td>
<td>4.07 ± 0.16</td>
<td>2.43 ± 0.01</td>
<td>0.26 ± 0.03t</td>
<td>0.03 ± 0.003</td>
<td>1.54t ± 0.12</td>
<td>0.08 ± 0.004</td>
</tr>
<tr>
<td>trauma 15 min (4 rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex</td>
<td>4.67 ± 0.07</td>
<td>2.82 ± 0.04</td>
<td>0.26 ± 0.02</td>
<td>0.03 ± 0.001</td>
<td>1.85 ± 0.14</td>
<td>0.15 ± 0.012</td>
</tr>
<tr>
<td>pons</td>
<td>4.03 ± 0.33</td>
<td>2.49 ± 0.04</td>
<td>0.25 ± 0.02</td>
<td>0.04 ± 0.003</td>
<td>1.50 ± 0.26</td>
<td>0.07 ± 0.008</td>
</tr>
<tr>
<td>medulla</td>
<td>4.18 ± 0.07</td>
<td>2.35 ± 0.07</td>
<td>0.20 ± 0.01</td>
<td>0.03 ± 0.002</td>
<td>1.27 ± 0.11</td>
<td>0.08 ± 0.009</td>
</tr>
</tbody>
</table>

*Magnitude of difference between control and experimental group: tP <0.05, tp <0.01, wP <0.001. For abbreviations see text.

MABP, arterial pO₂, pCO₂ and pH, were consistent in the normoxic groups. Various degrees of hypoxia and hypercapnia were observed in the animals with pulmonary edema.

**Cerebral Metabolic State**

The metabolic state obtained after 7 m/sec trauma is summarized in Table 2. Compared to the controls there were either no changes, or very discrete ones, showing up as a small decrease in PCr, and an increase in ADP and lactate in the brain-stem regions. There were no changes in the cerebral cortex and, in general, most animals sustained the concussion without detectable changes in energy state in the regions studied.

After a 9 m/sec trauma, the metabolic changes were far more pronounced and showed a characteristic course. In the majority of animals there was a marked decrease of PCr (Fig. 1), and increases of lactate (Fig. 2), ADP, and AMP (Fig. 3). In the pons, there was also a significant fall in ATP at all three times. The changes developed rapidly at the start of freezing 1 minute after the trauma, and tended to progress during the following few minutes. Thus, between 1 and 4 minutes there was a significant progression of the changes in the ATP and ADP concentrations and in the lactate/pyruvate ratio in the pons. However, the animals in the 4-minute group had quite different levels, as can be judged from the individual values of PCr shown in Fig. 1. Probably, some animals tended to normalize during the first few minutes, while others had started to deteriorate. Although a significant decrease of the mean ATP level was found only in the pons in the 4-minute group, about half of the animals showed a derangement of the energy state in the brain-stem regions to levels comparable to those obtained after 10 to 20 seconds of total ischemia, that is, levels associated with extinction of the electroencephalographic record and, evidently, loss of function. The 15-minute group showed a tendency toward normalization but, by necessity, this group represents a certain selection of surviving animals. The results emphasize that trauma could be accompanied by metabolite changes within a wide range. No significant change of the tissue glucose appeared at any time.

Regional differences were found at both trauma levels. After 7 m/sec trauma, the small changes found were localized in the brain-stem region. After 9 m/sec trauma, changes were seen in all regions, but 1 minute
after trauma the changes in the brain stem dominated. Thus, the changes of PCr, lactate, and the lactate/pyruvate ratio were significantly more severe in the pons and medulla than in the cortex. Four minutes after trauma these regional differences were not observed.

The groups of animals with hypoxia due to pulmonary edema generally showed more pronounced changes than the corresponding normoxic groups. In the two hypoxic animals of the 7 m/sec injury group at 1 minute, the decreases of PCr in cortex, and ATP in cortex and pons, were significantly lower (p = 0.036) than those found in the normoxic group. The single animal recorded at 4 minutes, however, did not differ from the normoxic ones. In the 9 m/sec injury group all parameters measured indicated a more severe energy derangement. The mean values are shown in Figs. 1–3. There were significant decreases of the glucose levels in most of the hypoxic groups.

Discussion

The general features of the trauma model have been discussed previously. The response to impact, in terms of blood pressure reaction, occurrence of pulmonary edema, mortality, and frequency of gross morphological changes (SAH), was very similar in the present material. Thus, the model seemed well standardized for the production of graded trauma reactions and suitable for assembling groups of animals for the analysis of brain metabolites.

As shown by the results obtained in the 7 m/sec trauma group and some of the 9 m/sec trauma animals, concussion could evidently be sustained without significant depletion of energy stores. The methods used, including delivery of trauma to the intact head and subsequent freezing in situ, did not permit freezing within standardized intervals shorter than 1 minute. In view of the results obtained it seems unlikely that more pronounced changes could have been present before that time.

The animals having a normal metabolic state all showed a typical concussion response. Previously, changes of neurophysiological function (in terms of altered excitatory state and transmission failure) have been reported in many experimental series. Apparently such changes occur in the presence of a normal metabolic state.

These data rule out the old theories of compression ischemia as the mechanism behind the concussive reaction. The concussion theory of Chason, et al. (see also Lindquist and LeRoy) of a mechanically induced subcellular derangement of energy-producing systems is also incompatible with these findings. On the other hand, these data do not exclude the possibility that a certain, small
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Fig. 2. Regional brain lactate (La, lower) and lactate/pyruvate ratio (La/Py, upper) in control and trauma groups (9 m/sec impact). Large Symbols: Mean values ± SEM of normoxic groups. Value significantly different from control values (p < 0.05 to 0.001) shown in black. * or ** indicate regional difference, that is, the change in the brain-stem region significantly more pronounced than that in the cortex (* = p < 0.05, ** = p < 0.01, paired t-test). Small Symbols: Mean values of corresponding hypoxic groups. Values of hypoxic group significantly higher than that of normoxic (p < 0.05 to 0.01) are shown in black. Number of animals within normoxic (N_N) and hypoxic (N_H) groups is given below graphs.

neuronal population was reversibly or permanently damaged. Such changes have been repeatedly found in experimental concussion.4,14,16,26 Glial reactions indicating primary nerve cell damage and axonal tearing have been reported in clinical concussion injuries.5,15,61

The deranged energy state found in the 9 m/sec injury group reflects an imbalance between energy production and utilization. Such changes appear both in conditions of an excessively increased metabolic rate, namely seizures, and when energy production is impaired by reduction of oxygen availability in hypoxia or ischemia.3,5,28,46 In head trauma either of these factors could contribute to derange energy metabolism. However, in the present 9 m/sec injury group, marked changes in metabolic state appeared as early as 1 minute after the trauma. Provided CBF is not critically reduced in this period, these data support the excitation theory of Walker, et al.53 It is unlikely that there should be a reduction of CBF during the main part of this early phase characterized by a high blood pressure. In previous studies of CBF in other models of experimental head injury, an immediate increase of flow, due to abolished autoregulation has been found.2,20,29,42 With the present model, acceleration with 7 m/sec impacts did not reduce regional CBF during the first minute.31 We conclude from these
findings that the deranged energy state must have been influenced by excitation and increased metabolic rate.

The findings of more pronounced changes in the brain stem than in the cortex at 1 minute support the concept of the present model as a concussion model predominantly affecting the brain stem. Following acceleration trauma, the physiological signs of excitation were short lasting, and had vanished within 2 minutes even in the 9 m/sec injury animals. Therefore, we tentatively explain the progressive derangement of the energy state from 1 to 4 minutes by the influence of a complicating factor, which might be ischemia. In support
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of this, angiographic studies showed vascular narrowing 1 to 20 minutes after the 9 m/sec trauma. Similar angiographic findings were reported by Rockoff and Ommaya, and McCullough, et al. In clinical studies arterial spasm has been increasingly recognized after head injury. Macpherson and Graham suggested vasospasm as one of the mechanisms behind posttraumatic ischemic brain lesions described. Increase of the intracranial pressure, reducing cerebral perfusion pressure, has also to be considered.

In the present series, there was a tendency toward normalization in the 15-minute group. This may reflect recovery after a short-lasting vasospasm, as shown in the angiographic study. However, irreversible brain damage may well be the end result of the 9 m/sec trauma.

It is well known that both the vascular trauma and the massive blood pressure response in concussion induce damage of the blood-brain barrier, leading to vasogenic edema. However, it is unlikely that such mechanisms could be rapid enough to trigger the development of energy failure during the first minutes after trauma. On the other hand, such factors may well be important in the later posttraumatic stages.

The unfavorable effect of hypoxia is evident and should be critical both in situations of high energy demand and in ischemia. Hypoxia is a common complication in head injury, and the treatment of respiratory failure is one of the main objects in clinical handling of head-injured patients.

Conclusions

The effects of the present trauma model as analyzed from the regional metabolic state of the brain tentatively imply that concussion is primarily a sudden change of neurophysiological function, probably entailing a high energy consumption but not necessarily inducing energy failure. Energy depletion and transmission failure may develop as consequences of 1) the concussion reaction per se, or 2) additional complicating factors such as hypoxia and ischemia. In the former case, the functional disturbance probably lasts only a few minutes. In the latter long-lasting coma and even permanent damage may occur. The functional and structural changes may involve several parts of the brain, but the brain-stem region is predominantly affected.

For evaluations of the possible mechanisms discussed, direct measurements of cerebral metabolic flux rate, and cerebral blood flow and oxygen consumption are discussed elsewhere.

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